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Molecular diversity of arbuscular mycorrhizal fungi and patterns of host association over time and space in a tropical forest

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Abstract

We have used molecular techniques to investigate the diversity and distribution of the arbuscular mycorrhizal (AM) fungi colonizing tree seedling roots in the tropical forest on Barro Colorado Island (BCI), Republic of Panama. In the first year, we sampled newly emergent seedlings of the understory treelet *Faramea occidentalis* and the canopy emergent *Tetragastris panamensis*, from mixed seedling carpets at each of two sites. The following year we sampled surviving seedlings from these cohorts. The roots of 48 plants were analysed using AM fungal-specific primers to amplify and clone partial small subunit (SSU) ribosomal RNA gene sequences. Over 1300 clones were screened for random fragment length polymorphism (RFLP) variation and 7% of these were sequenced. Compared with AM fungal communities sampled from temperate habitats using the same method, the overall diversity was high, with a total of 30 AM fungal types identified. Seventeen of these types have not been recorded previously, with the remainder being similar to types reported from temperate habitats. The tropical mycorrhizal population showed significant spatial heterogeneity and nonrandom associations with the different hosts. Moreover there was a strong shift in the mycorrhizal communities over time. AM fungal types that were dominant in the newly germinated seedlings were almost entirely replaced by previously rare types in the surviving seedlings the following year. The high diversity and huge variation detected across time points, sites and hosts, implies that the AM fungal types are ecologically distinct and thus may have the potential to influence recruitment and host composition in tropical forests.

Keywords: AM1, arbuscular mycorrhiza, community composition, diversity, SSU, tropical forest

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Introduction

The ecological importance and physiological benefits of arbuscular mycorrhizal (AM) fungi for their host plants have long been acknowledged (see Smith & Read 1997). Yet the apparent low global diversity of AM fungi compared with their associated plant communities has led to the belief that AM fungi are a homogeneous group and species are functionally redundant. However, recent experiments have demonstrated that individual AM fungal species may have a spectrum of effects on different host plants. In some cases the species that best promotes growth in one host may actually retard growth in another (Talukdar & Germida 1994; Streitwolf-Engel *et al*. 1997; van der Heijden *et al*. 1998a). Likewise, individual AM fungal species may differ in their own growth response to different plant species (Sanders & Fitter 1992; Bever *et al*. 1996; Eom *et al*. 2000). Consequently, the presence or absence of particular AM fungal species, as well as overall AM fungal diversity, can affect the productivity and diversity of host plant communities, both in experimental greenhouse studies (van der Heijden *et al*. 1998b; Klironomos *et al*. 2000) and in natural ecosystems (Gange *et al*. 1993; Newsham *et al*. 1995; Hartnett & Wilson 1999). Thus, it is becoming increasingly important to gain a better understanding of

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mycorrhizal diversity, and especially how it is organized in the field.

The diversity of AM fungi in the field has traditionally been estimated on the basis of spore counts. However, this method can be problematic because fungal sporulation rates are governed by many factors (Morton *et al*. 1995; Bever *et al*. 1996; Eom *et al*. 2000), and spores are not, therefore, a direct measure of diversity. Furthermore, the population of spores in the soil may bear little relation to the AM fungal population colonizing roots (Clapp *et al*. 1995). Advances in molecular techniques now make it feasible to directly identify the AM fungi *in planta* by using the polymerase chain reaction (PCR) to target specific AM fungal sequences. Several PCR-based methods have been developed over recent years, the majority of which target the ribosomal RNA (rDNA) genes (see Clapp *et al*. 2002 for review).

Even so, the analysis of ribosomal genes is not without its own problems when applied to field roots because any such approach is limited by the available genetic markers. Both Simon *et al*. (1992) and Helgason *et al*. (1998) designed primers for the SSU rDNA that would amplify all known glomalean fungi (VANS1 and AM1, respectively). However, new sequence data revealed that the VANS1 site is not well conserved in the Glomales (Clapp *et al*. 1999) and the AM1 site is absent from several highly diverged lineages (Redecker *et al*. 2000). Furthermore, the delineation of AM fungal species is ambiguous. The fungi are nonculturable, obligate symbionts and in some cases, individual spores have been found to contain multiple, genetically distinct nuclei (Kuhn *et al*. 2001). Although the more variable regions of the ribosomal genes can show large intraspecies variation (Sanders *et al*. 1995; LloydMacgilp *et al*. 1996; Clapp *et al*. 2001), differences between SSU sequences found within single spores are usually small compared with differences found among different AM fungal species (Clapp *et al*. 1999; Schussler *et al*. 2001a). Even so, this observation poses challenges to both the species concept in AM fungi and to the assessment of ecologically meaningful levels of genetic diversity.

Although molecular methods were first applied to AM research over 10 years ago (Simon *et al*. 1992), surprisingly few studies have focused on the mycorrhizal diversity *in planta* in the field. To date, the most extensive investigations have employed the AM1 primer. The multiple colonizations of AM fungi are separated into classes through cloning and PCR-restriction fragment length polymorphism (RFLP). The numbers of each class can then be used as an approximate estimate of their proportion in the root if it is assumed that the classes amplify and clone proportionally. Use of this method has revealed large differences between the diversity and population structure of AM fungi in different temperate ecosystems (Helgason *et al*. 1998, 2002; Daniell *et al*. 2001; Vandenkoornhuyse *et al*. 2002). Furthermore, these studies have provided evidence for nonrandom associations with host species or dominant vegetation types, and have shown the composition of AM fungi can change through space and time. These studies imply that the AM fungi are ecologically distinct and support the idea that individual AM fungi are important in ecosystem functioning.

All such studies have focused on temperate ecosystems, so very little is known about plant–AM fungal interactions in the extremely diverse communities found in a tropical rainforest. In contrast to most temperate tree species, which tend to form ectomycorrhizal associations, the vast majority of tropical tree species form arbuscular mycorrhizas (Smith & Read 1997). In phosphorous-limiting tropical soils, AM fungi have been shown to improve plant P-relations (Howeler *et al*. 1987; Siqueira *et al*. 1998) and increase the survival rate of many hosts (Janos 1980b), and may have differential growth effects on different hosts (Kiers *et al*. 2000). Nonetheless, much fundamental information is still lacking: AM fungal species identities, abundance and ecological roles are largely unknown. Thus we aimed to use molecular techniques to determine the diversity and identities of AM fungi in a tropical forest, and assess their distribution among different hosts, sites and time points.

Materials and Methods

Sampling

All samples were collected on Barro Colorado Island (BCI), a field station operated by the Smithsonian Tropical Research Institute in the Republic of Panama (9°10′ N, 79°51′ W), mean annual precipitation 2.6 m. Root samples from seedlings of *Faramea occidentalis* (L.) A. Rich and *Tetragastris panamensis* (Engl.) Kuntz were taken from mature forest at two sites, Balboa and Snyder Molino (≈ 2 km apart) in October 1998 and November 1999. Seedlings sampled in 1998 had recently germinated and still had their cotyledons attached. No new seedlings germinated the following year, therefore the seedlings sampled in 1999 were survivors from the 1998 cohort. For each sample, six seedlings of each species were randomly selected from a mixed carpet of the two species, from plots ≈ 15 m^2.

In addition, to provide context for the samples collected at these focal points, six seedlings of the pioneer species *Luehea seemannii* Triana & Planch. were sampled from a third site (Laboratory Clearing) in October 1998. This site is ≈ 1.5 km from the Snyder Molino site.

Molecular analysis of roots

DNA was extracted from plant roots using the potassium ethyl xanthate (PEX) method (Edwards *et al*. 1997). DNA extracts were further purified using Concert™ spin columns (Gibco BRL). Partial ribosomal small subunit (SSU) DNA

fragments were amplified using *Taq* DNA polymerase (Gibco BRL), a universal eukaryotic primer NS31 (Simon *et al*. 1992) and the primer AM1, designed to amplify AM fungal SSU sequences but not plant sequences (Helgason *et al*. 1998). Reactions were performed using 0.2 mM dNTPs, 10 pmol of each primer and the supplied reaction buffer to a final volume of 50 μL (PCR conditions: 94 °C for 3 min, 58 °C for 1 min, 72 °C for 1 min 30 s, then 24 cycles at 94 °C for 30 s, 58 °C for 1 min, 72 °C for 1 min 30 s). Products were cloned into pGEM-T Easy (Promega) and transformed into *Escherichia coli* (DH5α). Putative positive transformants were screened using a second NS31/AM1 amplification (PCR conditions: 24 cycles at 94 °C for 30 s, 58 °C for 1 min, 72 °C for 1 min 30 s). At least 20 positive clones from each sample were tested for RFLP by digestion with *Hin*fI and *Hsp*92II, according to the manufacturer's instructions (Promega). Examples of each RFLP type were reamplified using the vector primers SP6 and T7, purified using Concert™ spin columns, and sequenced on an ABI 377 automated sequencer using the dye terminator cycle sequencing kit with AmpliTaqFS DNA polymerase (Applied Biosystems). The sequencing primers were SP6 and T7.

Data analysis

Forward and reverse sequences were aligned and edited using LASERGENE SEQMAN (DNAStar Inc.). CLUSTALX (Thompson *et al*. 1997) was used for multiple alignment and neighbour-joining phylogenetic analyses (Saitou & Nei 1987), using *Corallochytrium limacisporum*, a putative choanozoan (Cavalier-Smith & Allsopp 1996) as the outgroup.

General loglinear modelling and multidimensional scaling were carried out using SPSS Version 10.0. Loglinear modelling explores the relationship between explanatory (time, site, host) and response (AM fungi) variables. A saturated model is constructed which, by definition, exactly reproduces the observed data. The relative importance of each variable or interaction may then be tested by removing terms from the model and calculating the likelihood ratio chi-square goodness-of-fit statistic. All models are hierarchical. The nine most frequent AM fungal types were used in the model. Multidimensional scaling indicates the degree of dissimilarity of the fungal communities colonizing each root, based on pairwise comparisons. To model dissimilarities, Euclidean distances were computed.

Diversity indices were computed using ESTIMATES (Version 5, RK Colwell, http://viceroy.eeb.uconn.edu/estimates).

Results

Identification of the AM fungal species

In the main study of *Faramea* and *Tetragastris* seedlings, a total of 1383 clones containing the SSU rRNA gene were investigated. All clones were analysed by *Hin*fI and *Hsp*92II RFLP typing, and 91 were sequenced. Examples of the sequences obtained are given in Fig. 1, with identical or near-identical sequences (< 3 bp difference) omitted for clarity. Based on the combined sequence and RFLP groupings, the clones have been classified into 30 AM fungal types. Most of the types are defined as sets of clones, each set having a unique RFLP pattern and forming a monophyletic clade. The exceptions are the Glo3, Glo14, Acau and Scut groupings. Although Glo3 and Glo14 each represent single RFLP patterns, they each comprise two distinct sequence clades, Glo3a and Glo3b, and Glo14a and Glo14b, respectively. The Acau 8, -9, -10 and Scut 1, -2, -3, -4 sequence groupings are not resolved by the enzymes used in this study, but these groupings have strong bootstrap support and show > 97% divergence therefore are indicated as separate types on the phylogeny (Fig. 1). However, for the purposes of all statistical analyses, only the RFLP groupings Glo3, Glo14, Acau and Scut are considered, resulting in a total of 23 AM fungal types (Table 1).

Because the primary method of grouping the AM fungal types is by their RFLP pattern, there is a large range in the level of sequence divergence within each type, from 0.04 to 4.70% (excluding Glo3 and Glo14). It is therefore possible that many of these groupings contain more than one AM species. For example, the grouping Glo8 contains sequences obtained from *Glomus intraradices* and *G. vesiculiferum* cultures. The AM fungal types classified here cannot be related precisely to either morphological or biological species, though we expect that both molecular and morphological methods underestimate the true AM fungal species diversity.

Of the 30 AM fungal types, 23 belong to the Glomaceae, 3 to the Acaulosporaceae and 4 to the Gigasporaceae. No AM fungal types from the Archaeosporaceae or the Paraglomaceae were detected, though this is expected because the AM1 primer does not match the SSU sequences of these taxa. Further, the AM1 primer contains two mismatches for sequences belonging to the *Glomus*-group B clade, as defined by Schussler *et al*. (2001b). These mismatches may be responsible for the apparent absence of these types from BCI. The majority of AM fungal types do not cluster closely with any sequences from AM fungi in culture, but the clades Glo1b, Glo3b and Glo8 include sequences with over 98% identity to the sequences for *G. mosseae* (Z14007), *G. sinuosum* (AJ133706) and *G. intraradices* (X58725), respectively. Seventeen of the AM fungal types are novel and might be unique to the tropics, whilst the remainder are closely related to types detected in temperate habitats (see Helgason *et al*. 1998; Vandenkoornhuyse *et al*. 2002). Examples of the most closely related sequences are shown in Fig. 1, and these include instances of SSU sequence identity between temperate and tropical AM types.

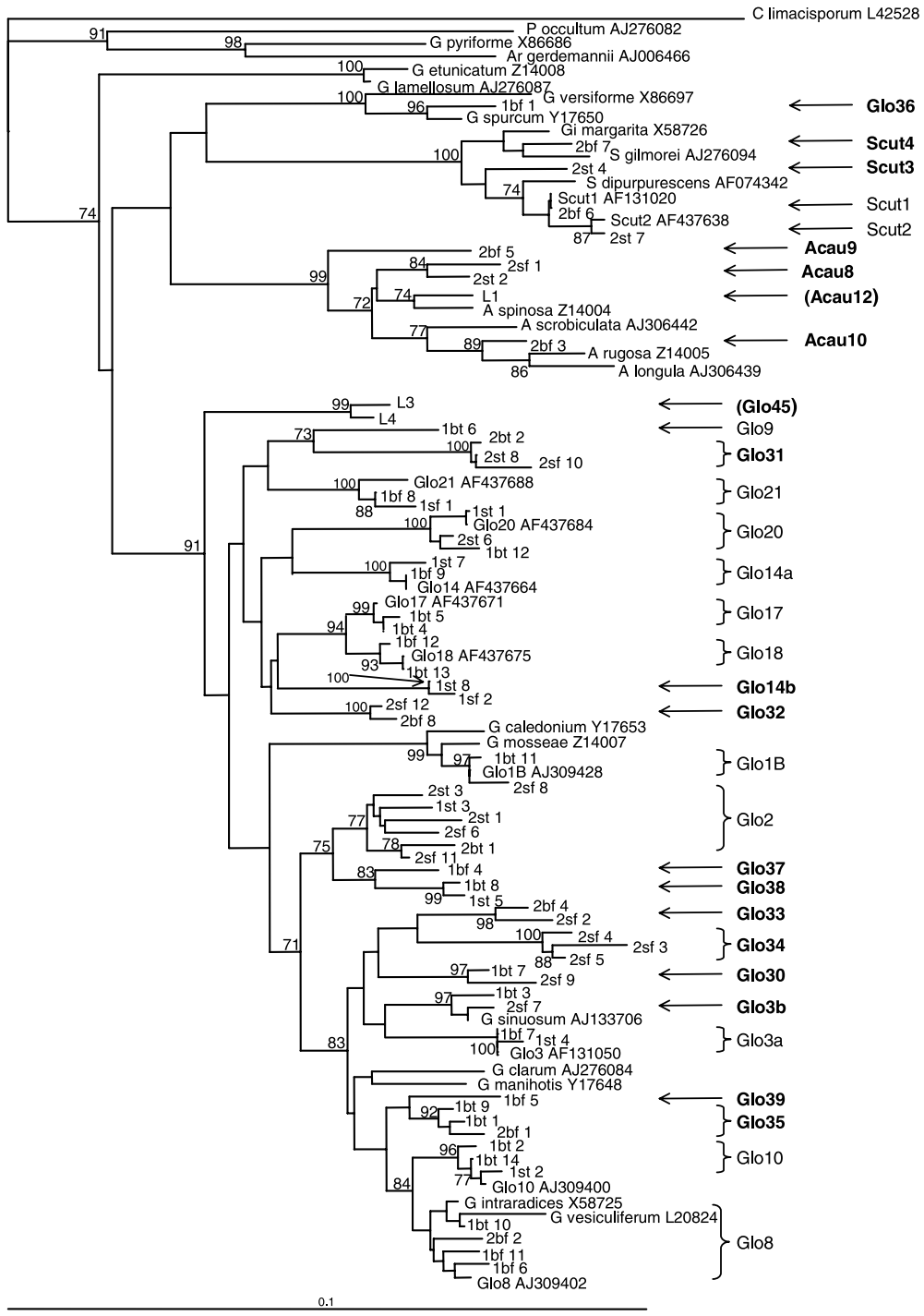

Fig. 1 Neighbour-joining phylogenetic tree showing examples of the sequence types colonizing seedlings in a tropical forest. Bootstrap values > 70% (1000 replicates) are shown. Individual clones are identified by time point (1 = October 1998, 2 = November 1999), site (b = Balboa, s = Snyder Molino) and host (f = *Faramea*, t = *Tetragastris*). L = clones from *Luehea* in Laboratory Clearing, October 1998. Sequences that have been detected in temperate field studies (Helgason *et al*. 1998; Daniell *et al*. 2001; Vandenkoornhuyse *et al*. 2002) are included, with their accession numbers, only where they are closely related to sequences from this study. Group identifiers (for example Glo3) are consistent with the temperate field studies and correspond to those used in the text. Group identifiers in parentheses denote sequences found only in *Luehea* seedlings. AM fungal types unique to this study are shown in bold. All new sequences have been submitted to the international databanks under accession numbers AY129570-AY129633.

Table 1 Number of clones of each random fragment length polymorphism (RFLP) type obtained for each time point, site, host combination. Each row represents the sum from six seedlings. Identified by time point (1 = October 1998, 2 = November 1999), site (b = Balboa, s = Snyder Molino, m = Miller) and host (f = *Faramea*, t = *Tetragastris*). The final row (L) shows the number of clones of each RFLP type detected colonizing Luehea. The mean and standard deviation of the number of AM fungal types detected per individual seedling is indicated in the last two columns

	Glo1b	Glo30	Glo35	Glo2	Glo8	Glo3	Glo18	Glo10	Glo20	Glo33	Glo14	Glo17	Glo21	Glo 1	Glo38	Glo34	Glo32	Glo39	Glo9	Glo36	Glo37	Glo45	Acau	Scut	Total	$\bar{X}$	SD
1bf	60	2	13	—	33	3	29	32	1	—	1	—	–	—	1	—	—	2	—	1	1	—	—	—	179	6.18	0.96
1bt	17	22	23	9	29	1	14	11	7	—	—	4	–	—	7	—	—	—	1	—	—	—	—	—	145	7.16	1.86
1sf	166	—	8	1	4	1	—	—	1	—	9	5	9	—	—	—	—	—	—	—	—	—	—	—	204	4.64	1.44
1st	122	5	12	4	11	4	13	8	1	—	10	—	1	—	1	—	—	—	—	—	—	—	—	—	192	5.32	2.61
2bf	—	66	42	27	2	10	1	1	2	5	—	6	—	2	—	—	1	—	—	—	—	—	8	17	190	6.54	1.85
2bt	10	82	16	27	3	17	2	—	5	2	—	1	—	1	—	—	—	—	—	—	—	—	—	—	166	5.28	1.14
2sf	3	13	33	46	1	19	1	—	5	10	—	1	—	3	—	3	1	—	—	—	—	—	3	1	143	6.52	1.83
2st	3	8	39	41	2	6	—	2	26	12	—	—	1	3	—	—	—	—	—	—	—	—	4	17	164	5.56	2.12
L	28	22	3	3	8	14	1	2	19	1	6	2	—	3	1	—	—	—	—	1	17	3	19	—	153	7.12	1.1
Total	381	198	186	155	85	61	60	54	48	29	20	17	11	9	9	3	2	2	1	1	1	—	15	35	1383	5.52	1.88

In the *Luehea* study, 153 clones were analysed which have been classified into 18 AM fungal types, using the same approach as above. Two of these types, Glo45 and Acau 12, are unique to the *Luehea* seedlings, and their sequences are indicated in Fig. 1. The remaining types were also found colonizing the *Faramea* and *Tetragastris* seedlings (Table 1).

AM fungal distribution

Through time. The most dramatic difference in the AM fungal population colonizing *Faramea* and *Tetragastris* seedlings occurred over time (Table 1). In October 1998, the AM fungal population was heavily dominated by the Glo1b type (50%), with moderate colonization by Glo8, Glo10, Glo18 and Glo35. In contrast, with the exception of Glo35, these types had vanished almost completely from the population a year later. The November 1999 population was not dominated by any single AM fungal type, with Glo30 (25%) Glo2 (21%) and Glo35 (19%) being the most numerous. Many of the types present in October 1998 were not detected the following year (e.g. Glo38 and Glo14), whilst many new types were found (e.g. Glo33, Acau and Scut).

Despite the dominance of Glo1b in the first year, the overall fungal diversity and evenness were higher in October 1998 than November 1999 (Shannon diversity H, and evenness E, indices: $H = 2.12$, $E = 0.75$ and $H = 1.88$, $E = 0.66$, respectively). The average number of AM fungal types colonizing each root also declined between the time points (October 1998 mean = 7.04, SD = 1.62; November 1999 mean = 6.02, SD = 1.76). The large shift in the AM fungal population over time is evidenced by the multidimensional scaling (MDS) plot in which, with the exception of a few outliers, the two populations are well separated (Fig. 2). Although loglinear modelling does not take into account the degree of variation between replicates, the MDS plot indicates that the differences between the predictive variables is greater than the variation between them. Accordingly, loglinear modelling was used to explore the relationship between the explantory (time, site, host) and response (AM fungi) variables, and reveals highly significant differences between the AM fungal populations in October 1998 and November 1999 (Table 2 model 1a).

A separate study on BCI followed the course of the mycorrhizal population colonizing a cohort of *Tetragastris* seedlings over three years at a different site, Miller (Husband *et al*. 2002). Although the three sites are significantly different in terms of their AM population, a comparison of the change in abundance of the most common AM fungal types reveals a strikingly similar trend (Fig. 3). At all three sites in all hosts, previously rare AM fungal types replace the types dominant in the newly emerged

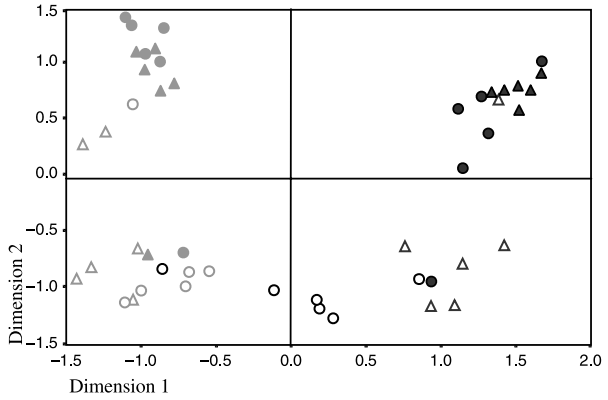

Fig. 2 Multidimensional scaling plot indicating the degree of similarity among the AM fungal communities colonizing each seedling. The greater the similarity between roots, the smaller the distance between points on the plot (black symbols = October 1998, grey symbols = November 1999; open symbols = Balboa, closed symbols = Snyder Molino; triangles = *Faramea*, circles = *Tetragastris*).

Table 2 Likelihood ratios (LR) produced by general loglinear analysis. The table shows the LR produced by the model when the specified term is removed. Higher LR values indicate greater deviation from the saturated model

	Model	LR	d.f.	*P*
1a	Time main effect	985	32	< 0.001
1b	Site main effect	445	32	< 0.001
1c	Host main effect	189	32	< 0.001
1d	Host*Time interaction	80	16	< 0.001
1e	Host*Site interaction	76	16	< 0.001
1f	Site*Time interaction	66	16	< 0.001
1g	Host*Site*Time interaction	34	8	< 0.001

seedlings. For all host–site combinations, Glo1b and Glo8 decrease in abundance over time, whereas Glo30 increases. The proportion of the AM fungal type Glo35 fluctuates between time points at all sites but does not change significantly at any of them. The only trend that is not uniform across all sites and hosts is the shift in Glo2. At the Balboa and Snyder Molino sites this AM type increases in abundance in the surviving seedlings, but at the Miller site it decreases in abundance (see Fig. 3 and Husband *et al*. 2002).

Through space. The mycorrhizal communities differ greatly between the two main study sites. For example, in October 1998 there is a much higher proportion of Glo1b, Glo14 and Glo21 in the population at Snyder Molino, whereas Glo8, Glo10 and Glo18 are more prevalent at Balboa (Table 1). The MDS plot reveals this variation by clearly separating the two sites (Fig. 2). Although there are exceptions, most of the roots from Snyder Molino (closed symbols) are distributed towards the top of the plot, whereas the majority of roots from Balboa (open symbols) are towards the bottom. The difference between the two sites is highly significant (Table 2 model 1b).

Although the loglinear analysis shows there is a significantly different AM fungal population colonizing the two hosts *Faramea* and *Tetragastris* (Table 2 model 1c), a comparison of likelihood ratios indicates that both time and site have a greater influence on the AM fungal population than host species alone. However, the two host interaction terms are very strong (Table 2 model 1d, 1e), which implies that within a site the two hosts are colonized by significantly different AM fungal populations, and that the change in AM fungal population over time is different for each host. In essence there are highly significant, nonrandom associations between the different hosts and AM fungi. For example, at Balboa in the first year there was a higher proportion of Glo1b and a lower proportion of Glo30 colonizing *Faramea* than *Tetragastris*. At the same site the following year, *Faramea* was colonized by a higher proportion of Glo35 and Scut than *Tetragastris*.

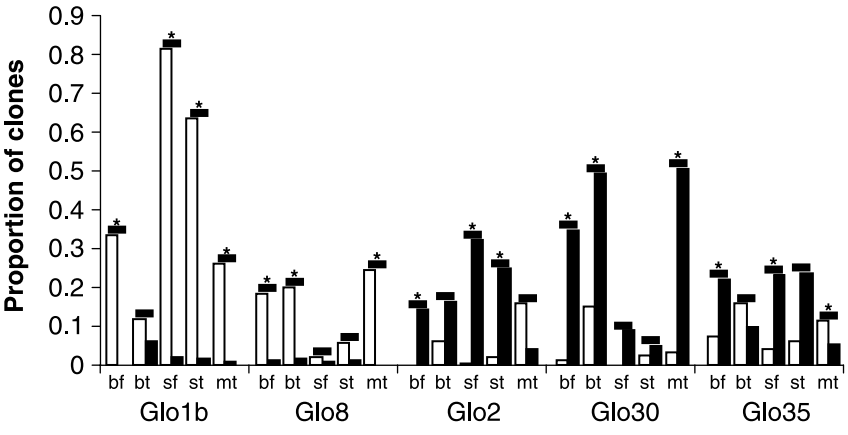

Fig. 3 Paired columns show, for the two time points (white columns = October 1998, black columns = November 1999), the proportion of the five most abundant AM fungal types colonizing seedlings for each combination of site (b = Balboa, s = Snyder Molino, m = Miller) and host (f = *Faramea*, t = *Tetragastris*). Data for the Miller site are taken from Husband *et al*. (2002). Asterisks denote significantly different mean ranks between the time points ($P < 0.05$ by Mann–Whitney *U*-test).

Discussion

AM fungal diversity is high

Tropical forests are famous for their high plant diversity. Thus, the finding of 30 AM fungal types, which could potentially equate to a fifth of the world's described AM fungal species, suggests that tropical mycorrhizal diversity is similarly high. Even so, the true ecological diversity is almost certainly higher than recorded here. Two new AM fungal types were detected in the roots of *Luehea*, indicating that the choice of site and host does indeed limit the fungal diversity recorded. Furthermore, not only will the AM1 primer exclude highly divergent lineages, but RFLP groupings have a low genetic resolution, therefore some of the AM fungal types described here may consist of more than one species (for example Glo8). Equally, the opposite is possible because multiple SSU sequences can exist within single spores (Clapp *et al*. 1999; Schussler *et al*. 2001a). However, the level of intraspecies SSU divergence is usually of the same magnitude as the variation within the groupings reported here, therefore it is highly unlikely that we have overestimated the AM fungal diversity. All the same, we do not know enough about the relationship between SSU and ecological diversity to fully interpret the meaning of these 30 AM fungal types. Accordingly, it is also difficult to assess the full implications of the high levels of sequence similarity detected between the temperate and tropical habitats. Specifically, we are uncertain whether our results reflect a true global distribution of AM fungal types (as reported for many spore morphotypes) or simply that the SSU is relatively conserved.

A comparison of the diversity we observed, with that recorded from other studies using the same method, reveals that the Shannon diversity index is appreciably higher for the mycorrhizal community on BCI ($H = 2.33$, $H_{max} = 3.135$, based on 48 roots from 2 host species) compared with a temperate grassland ($H = 1.71$, $H_{max} = 2.890$, based on 47 roots from 2 host species, Vandenkoornhuyse *et al*. 2002), a seminatural woodland ($H = 1.44$, $H_{max} = 2.565$, based on 49 roots from 5 host species, Helgason *et al*. 1998) or temperate arable fields ($H = 1.16$, $H_{max} = 2.08$, based on 79 roots from 4 host species, calculated from Daniell *et al*. 2001). Interestingly, although the diversity index is much higher for the tropical community, the actual 'species' number is not so high, compared with the 24 phylotypes detected in the temperate grassland. Although it is possible that relative to the grassland the tropics have an unusually low mycorrhizal richness, it seems more likely that the lower sampling intensity of this tropical study (over 40% more clones per root were analysed by Vandenkoornhuyse *et al*.) and more conservative criteria for identifying groups have led to the true AM fungal richness being underestimated in the present study.

Taken as a whole, we have shown that this tropical mycorrhizal community is highly diverse, but we believe the true diversity to be greater still. We support the opinion of other authors (Bever *et al*. 2001; Helgason *et al*. 2002) who have challenged the long-held view that AM fungi are depauperate relative to plant communities, and expect that in time many more AM fungal species will be discovered.

AM community changes through space

The mycorrhizal community on BCI mirrors the plant community in two important aspects: both are highly diverse and both have a patchy distribution. The large difference between the AM fungal communities at the two sites may reflect variation in soil factors such as pH, nutrient content, moisture and temperature, which are known to influence spore distributions (Koske 1987; Porter *et al*. 1987; Johnson *et al*. 1991; Cuenca & Meneses 1996). At present we do not know the mechanism maintaining the nonrandom associations between different hosts and AM fungi, although it has been reported that fungal sporulation rates vary under different host species, implying a host effect (Sanders & Fitter 1992; Bever *et al*. 1996; Eom *et al*. 2000). There is also some experimental evidence that individual AM fungi exhibit a preference for particular hosts (Giovannetti & Hepper 1985; McGonigle & Fitter 1990; Talukdar & Germida 1994; Helgason *et al*. 2002).

We believe the nonrandom association between different hosts and fungi detected on BCI, is evidence for site-dependent host preference. The two hosts involved, *Tetragastris* and *Faramea*, have distinct life histories. *Tetragastris* is a mid-to-late successional species associated with mature forest, whereas *Faramea* is a persistent understory species. With such contrasting ecological strategies it is likely that they differ physiologically, and that ecologically distinct AM fungi preferentially colonize one host over the other. Alternatively, it could be argued that although the roots were sampled from a mixed carpet of seedlings, the two species germinated at different times when different AM fungi were available. Yet nonrandom associations between host and symbiont were detected at both time points (i.e. in the three month old seedlings and the surviving 16-month-old seedlings the following year), despite an overall shift in the fungal population. Such persistent yet dynamic nonrandom associations are evidence for host preference, albeit one that is heavily influenced by edaphic conditions.

The partitioning of AM fungi into different abiotic (site) and biotic (host) niches has important implications for ecosystem functioning as a whole. First, niche partitioning would enable ecologically distinct AM fungi to coexist and may contribute to the high fungal diversity on BCI. Second, different distributions of AM fungi may have the potential to influence plant community structure. Janos (1980a) hypothesized that a patchy mycorrhizal distribution

influences tropical succession because obligately mycorrhizal species survive to maturity only where AM fungi are present. This effect does not depend on functional diversity within the AM fungal community. However, recent pot experiments have demonstrated that AM fungi have differential effects on different hosts (Talukdar & Germida 1994; van der Heijden *et al*. 1998a, b; Helgason *et al*. 2002). If it could be demonstrated that the nonrandom associations between different hosts and AM fungi on BCI affect the successful recruitment of seedlings, then we would have evidence both of host preference, and a mechanism by which mycorrhiza may influence the entire above-ground community structure.

AM community changes through time

Additional support that the mycorrhiza on BCI may influence plant diversity comes from the huge shift in the mycorrhizal population over time. The simplest explanation for these changes is that ecologically distinct AM fungi respond differently to changes in the abiotic environment, and as the environment changes over time, so do the dominant AM fungal species. Such responses, plus changes in host phenology, are the implicit assumptions used previously to explain temporal variation in mycorrhizal communities (Lee & Koske 1994; Merryweather & Fitter 1998; Eom *et al*. 2000; Daniell *et al*. 2001). Uniquely, we have information over two successive years for the mycorrhizal population colonizing the same cohort of seedlings. Therefore, changes in the biotic environment may also influence the mycorrhizal population. Indeed in a study at a different site on BCI we found that significantly different fungal populations colonized two age classes of seedlings sampled at the same time point (Husband *et al*. 2002). This indicates that the fungal community may be influenced by the age or history of the host.

Both this study and that of Husband *et al*. (2002) show a strong repeating pattern, whereby the dominant mycorrhizal types are replaced by previously rare types in the surviving seedlings (a similar group of types in both cases, see Fig. 3). Furthermore, both studies reveal a decrease in fungal evenness and diversity across the time points. These trends, together with the potential influence of host age, suggest that two contrasting but not necessarily mutually exclusive processes may contribute to the shift in fungal types we detected. Either there is a succession of AM fungal types within a single host, driven by differences in fungal life-history strategies (see Hart *et al*. 2001 for review); or individual AM fungi affect seedling recruitment, so that the most effective host-fungus combination has a higher probability of survival and is consequently enriched in the surviving population.

In a pot experiment in which different tropical tree seedlings were cross-inoculated with AM fungal inocula from different sources, Kiers *et al*. (2000) showed that conspecific seedlings and adults may gain maximum growth benefit from associations with different AM fungi. If this is a true mycorrhizal effect that occurs in the field, it would cause the community composition of the AM fungi to change as the seedlings mature. In addition, seeds that disperse away from their parent would have a greater probability of encountering mycorrhiza most beneficial for survival. Thus AM fungi may also affect plant communities by influencing negative density-dependent recruitment of seedlings, which has been shown to enhance tropical tree diversity (Harms *et al*. 2000).

It is becoming abundantly clear that the community of AM fungi in plant roots varies widely from place to place, from time to time and from host to host. This variation is itself proof that the different fungal types are not ecologically equivalent. The challenge for the future is to identify the important functional differences among AM fungi.

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References

Bever JD, Morton JB, Antonovics J, Schultz PA (1996) Host-dependent sporulation and species diversity of arbuscular mycorrhizal fungi in a mown grassland. *Journal of Ecology*, **84**, 71–82.

Bever JD, Schultz PA, Pringle A, Morton JB (2001) Arbuscular mycorrhizal fungi: more diverse than meets the eye, and the ecological tale of why. *Bioscience*, **51**, 923–931.

Cavalier-Smith T, Allsopp M (1996) *Corallochytrium*, an enigmatic non-flagellate protozoan related to choanoflagellates. *European Journal of Protistology*, **32**, 306–310.

Clapp JP, Fitter AH, Young JPW (1999) Ribosomal small subunit sequence variation within spores of an arbuscular mycorrhizal fungus, *Scutellospora* sp. *Molecular Ecology*, **8**, 915–921.

Clapp JP, Helgason T, Daniell TJ, Young JPW (2002) Genetic studies of the structure and diversity of arbuscular mycorrhizal communities. In: *Mycorrhizal Ecology* (eds van der Heijden MGA, Sanders IR), pp. 201–224. Springer Verlag, Heidelberg.

Clapp JP, Rodriguez A, Dodd JC (2001) Inter- and intra-isolate rRNA large subunit variation in *Glomus coronatum* spores. *New Phytologist*, **149**, 539–554.

Clapp JP, Young JPW, Merryweather JW, Fitter AH (1995) Diversity of fungal symbionts in arbuscular mycorrhizas from a natural community. *New Phytologist*, **130**, 259–265.

Cuenca G, Meneses E (1996) Diversity patterns of arbuscular mycorrhizal fungi associated with cacao in Venezuela. *Plant and Soil*, **183**, 315–322.

Daniell TJ, Husband R, Fitter AH, Young JPW (2001) Molecular diversity of arbuscular mycorrhizal fungi colonising arable crops. *FEMS Microbiology Ecology*, **36**, 203–209.

Edwards SG, Fitter AH, Young JPW (1997) Quantification of an arbuscular mycorrhizal fungus, *Glomus mosseae*, within plant roots by competitive polymerase chain reaction. *Mycological Research*, **101**, 1440–1444.

Eom AH, Hartnett DC, Wilson GWT (2000) Host plant species effects on arbuscular mycorrhizal fungal communities in tallgrass prairie. *Oecologia*, **122**, 435–444.

Gange AC, Brown VK, Sinclair GS (1993) Vesicular-arbuscular mycorrhizal fungi — a determinant of plant community structure in early succession. *Functional Ecology*, **7**, 616–622.

Giovannetti M, Hepper CM (1985) Vesicular arbuscular mycorrhizal infection in *Hedysarum-Coronarium* and *Onobrychis-Viciaefolia* — host endophyte specificity. *Soil Biology and Biochemistry*, **17**, 899–900.

Harms KE, Wright SJ, Calderon O, Hernandez A, Herre EA (2000) Pervasive density-dependent recruitment enhances seedling diversity in a tropical forest. *Nature*, **404**, 493–495.

Hart MM, Reader RJ, Klironomos JN (2001) Life-history strategies of arbuscular mycorrhizal fungi in relation to their successional dynamics. *Mycologia*, **93**, 1186–1194.

Hartnett DC, Wilson GWT (1999) Mycorrhizae influence plant community structure and diversity in tallgrass prairie. *Ecology*, **80**, 1187–1195.

van der Heijden MGA, Boller T, Wiemken A, Sanders IR (1998a) Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. *Ecology*, **79**, 2082–2091.

van der Heijden MGA, Klironomos JN, Ursic M *et al.* (1998b) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature*, **396**, 69–72.

Helgason T, Daniell TJ, Husband R, Fitter AH, Young JPW (1998) Ploughing up the wood-wide web? *Nature*, **394**, 431–431.

Helgason T, Merryweather JW, Denison J *et al.* (2002) Selectivity and functional diversity in arbuscular mycorrhizas of co-occuring fungi and plants from a temperate deciduous woodland. *Journal of Ecology*, **90**, 371–384.

Howeler RH, Sieverding E, Saif S (1987) Practical aspects of mycorrhizal technology in some tropical crops and pastures. *Plant and Soil*, **100**, 249–283.

Husband R, Herre EA, Young JPW (2002) Temporal variation in the arbuscular mycorrhizal communities colonising seedlings in a tropical forest. *FEMS Microbiolgy Ecology*, **42**, 131–136.

Janos DP (1980a) Mycorrhizae influence tropical succession. *Biotropica*, **12**, 56–64.

Janos DP (1980b) Vesicular–arbuscular mycorrhizae affect lowland tropical rain forest plant growth. *Ecology*, **61**, 151–162.

Johnson NC, Zak DR, Tilman D, Pfleger FL (1991) Dynamics of vesicular–arbuscular mycorrhizae during old field succession. *Oecologia*, **86**, 349–358.

Kiers ET, Lovelock CE, Krueger EL, Herre EA (2000) Differential effects of tropical arbuscular mycorrhizal fungal inocula on root colonization and tree seedling growth: implications for tropical forest diversity. *Ecology Letters*, **3**, 106–113.

Klironomos JN, McCune J, Hart M, Neville J (2000) The influence of arbuscular mycorrhizae on the relationship between plant diversity and productivity. *Ecology Letters*, **3**, 137–141.

Koske RE (1987) Distribution of a mycorrhizal fungi along a latitudinal temperature-gradient. *Mycologia*, **79**, 55–68.

Kuhn G, Hijri M, Sanders IR (2001) Evidence for the evolution of multiple genomes in arbuscular mycorrhizal fungi. *Nature*, **414**, 745–748.

Lee PJ, Koske RE (1994) *Gigaspora-Gigantea* — seasonal abundance and aging of spores in a sand dune. *Mycological Research*, **98**, 453–457.

LloydMacgilp SA, Chambers SM, Dodd JC *et al.* (1996) Diversity of the ribosomal internal transcribed spacers within and among isolates of *Glomus mosseae* and related mycorrhizal fungi. *New Phytologist*, **133**, 103–111.

McGonigle TP, Fitter AH (1990) Ecological specificity of vesicular arbuscular mycorrhizal associations. *Mycological Research*, **94**, 120–122.

Merryweather J, Fitter A (1998) The arbuscular mycorrhizal fungi of *Hyacinthoides nonscripta* — II. Seasonal and spatial patterns of fungal populations. *New Phytologist*, **138**, 131–142.

Morton JB, Bentivenga SP, Bever JD (1995) Discovery, measurement, and interpretation of diversity in arbuscular endomycorrhizal fungi (Glomales, Zygomycetes). *Canadian Journal of Botany — Revue Canadienne de Botanique*, **73**, S25–S32.

Newsham KK, Fitter AH, Watkinson AR (1995) Multi-functionality and biodiversity in arbuscular mycorrhizas. *Trends in Ecology and Evolution*, **10**, 407–411.

Porter WM, Robson AD, Abbott LK (1987) Field survey of the distribution of vesicular arbuscular mycorrhizal fungi in relation to soil-pH. *Journal of Applied Ecology*, **24**, 659–662.

Redecker D, Morton JB, Bruns TD (2000) Ancestral lineages of arbuscular mycorrhizal fungi (Glomales). *Molecular Phylogenetics and Evolution*, **14**, 276–284.

Saitou N, Nei M (1987) The neighbor-joining method — a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, **4**, 406–425.

Sanders IR, Alt M, Groppe K, Boller T, Wiemken A (1995) Identification of ribosomal DNA polymorphisms among and within spores of the Glomales — application to studies on the genetic diversity of arbuscular mycorrhizal fungal communities. *New Phytologist*, **130**, 419–427.

Sanders IR, Fitter AH (1992) Evidence for differential responses between host fungus combinations of vesicular arbuscular mycorrhizas from a grassland. *Mycological Research*, **96**, 415–419.

Schussler A, Gehrig H, Schwarzott D, Walker C (2001a) Analysis of partial Glomales SSU rRNA gene sequences: implications for primer design and phylogeny. *Mycological Research*, **105**, 5–15.

Schussler A, Schwarzott D, Walker C (2001b) A new fungal phylum, the *Glomeromycota*: phylogeny and evolution. *Mycological Research*, **105**, 1413–1421.

Simon L, Lalonde M, Bruns TD (1992) Specific smplification of 18*S* fungal ribosomal genes from vesicular–arbuscular endomycorrhizal fungi colonizing roots. *Applied and Environmental Microbiology*, **58**, 291–295.

Siqueira JO, Carneiro MAC, Curi N, Rosado SCS, Davide AC (1998) Mycorrhizal colonization and mycotrophic growth of native woody species as related to successional groups in Southeastern Brazil. *Forest Ecology and Management*, **107**, 241–252.

Smith SE, Read DJ (1997) *Mycorrhizal Symbiosis*, 2nd edn. Academic Press, San Diego.

Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR (1997) Clonal

growth traits of two *Prunella* species are determined by co-occurring arbuscular mycorrhizal fungi from a calcareous grassland. *Journal of Ecology*, **85**, 181–191.

Talukdar NC, Germida JJ (1994) Growth and yield of lentil and wheat inoculated with 3 *Glomus* isolates from Saskatchewan soils. *Mycorrhiza*, **5**, 145–152.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, **25**, 4876–4882.

Vandenkoornhuyse P, Husband R, Daniell TJ *et al.* (2002) Arbuscular mycorrhizal community composition associated with two plant species in a grassland ecosystem. *Molecular Ecology*, **11**, 1555–1564.

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